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# Table of Contents

# Development of the Replica Barcode Selection Technology for the Study of Breast Cancer Metastasis.

## INTRODUCTION

### Introduction

An emerging view of cancer metastasis posits that, for at least some forms of cancer, a small subset of cells in the primary tumor might have much greater potential than other cells to metastasize and give rise to secondary tumors. In this proposal, we will refer to such cells as metastasis-capable cells (MCCs). MCCs may include cancer stem cells, defined as the subset of tumor cells that behave like stem cells in that they are capable of self-renewal as well as differentiation into all cell types of the tumor. But MCCs may also include cells that are metastatically potent but unable to differentiate into all cell types of the tumor.

It is important to study MCCs given their essential role in metastasis. A useful approach for studying MCCs is to isolate them in order to characterize their properties in isolation from other cells in the tumor. Furthermore, it is preferable to isolate MCCs in a prospective manner whereby the isolated cells have not yet gone through metastasis despite having the potential to do so. This is important because the process of metastasis could significantly alter the genetic/epigenetic properties of MCCs.

The current method for the prospective isolation of MCCs, including the isolation of cancer stem cells, relies on the expression of certain markers. However, for many cancers, it is not known which markers are good indicators of metastatic potential or even whether such markers exist. Ideally, therefore, one would like to carry out the prospective isolation of MCCs based on function – *i.e.*, their ability to metastasize – rather than their marker expression.

Therein lies a dilemma: how to isolate cells based on their metastatic ability while also avoid subjecting the cells to metastasis? Here, we propose to develop a technology intended to circumvent this dilemma. Termed the “replica barcode selection” (RBS) assay, this technology is designed to prospectively isolate cells that may be predisposed to undergo metastasis, but do so without actually letting the cells go through metastasis.

The RBS assay relies on the assumption that, if a cancer cell has a predisposition to undergo metastasis, then this predisposition is likely inherited by its daughter cells when this cell divides. The general scheme of RBS is as follows. First, the starting population of cancer cells is modified in a manner that accords each cell with a unique genetic identifier, or “barcode”. Next, these cells are expanded *in vitro* such that each cell carrying its unique barcode is amplified into multiple daughter cells carrying that same barcode. Cells are then split into two pools: a test pool and a reserve pool. The test pool is transplanted into mice to form primary tumors, followed by further incubation to allow the development of secondary tumors. Barcodes from individual secondary foci are then read, and for each barcode identified this way, cells in the reserve pool bearing this same barcode will be retrieved. These cells are the sisters of – and may therefore possess similar metastatic potential as – the MCCs in the test pool that have actually undergone metastasis.

Our proposal has two specific aims. The first is the development of the RBS assay, including the construction and validation of all the components of the assay. The second aim is the application of the assay to the study of breast cancer metastasis. Specifically, we will attempt to use the RBS assay to isolate MCCs from a mouse model of breast cancer metastasis.

This proposal provides an innovative technology for the prospective isolation of a key subpopulation of tumor cells – those that may be predisposed to undergo metastasis. If successfully developed, the technology should greatly facilitate the mechanistic understanding of cancer metastasis. When applied to the study of breast cancer metastasis, the proposal could ultimately lead to better diagnosis and treatment.

# BODY

## Body

In the first year of funding, the focus of our work was the first of the two specific aims of the proposal, namely, to develop the Replica Barcode Selection (RBS) assay, including the construction and validation of all the components of the assay. We built a lentiviral vector expressing the GFP reporter under the constitutively active human EF1A promoter, with the open reading frame of GFP flanked on both 5' and 3' ends by barcode sequences (Figure 1). This vector also expresses the puromycin resistance gene. To validate the vector, we transfected it into the 293T packaging cells, obtained infectious viral particles in the supernatant, and used it to transduce the mouse breast cancer cell line TM40D-MB-Luc. Transduced cells were selected with puromycin. We observed the appropriate level of GFP fluorescence signal (Figure 2). The signal was significantly reduced when 5' or 3' of GFP is targeted for knockdown. We now have in hand a library of lentiviral vectors containing randomized barcodes, and a corresponding library of infectious lentiviral particles. The TM40D-MB-Luc cells transduced with the lentivirus library have been transplanted into mouse models to test the utility of the RBS assay to identify metastasis-capable cells in an *in vivo* setting. To the present, we have already identified and harvested tumor cells from the metastatic organs, lung and bone. Tumor cells were subcloned to be decoded by the Bruce Lahn laboratory.

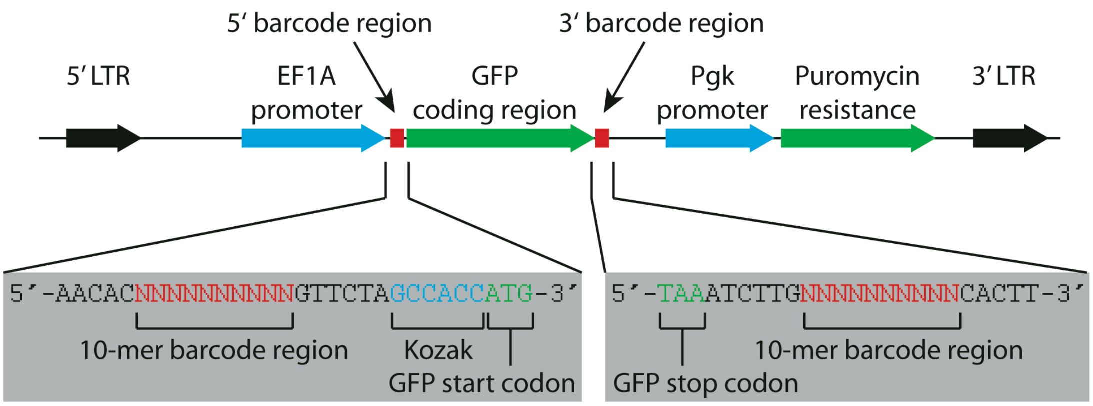



Figure 1. Schematic diagram of the lentiviral vector. The vector contains a human EF1A promoter driving the GFP coding region. GFP is flanked on both 5' and 3' ends by barcode sequences. Each barcode sequence consists of a 10-mer random sequence. The vector also contains a mouse Pgk promoter driving the puromycin resistance gene.

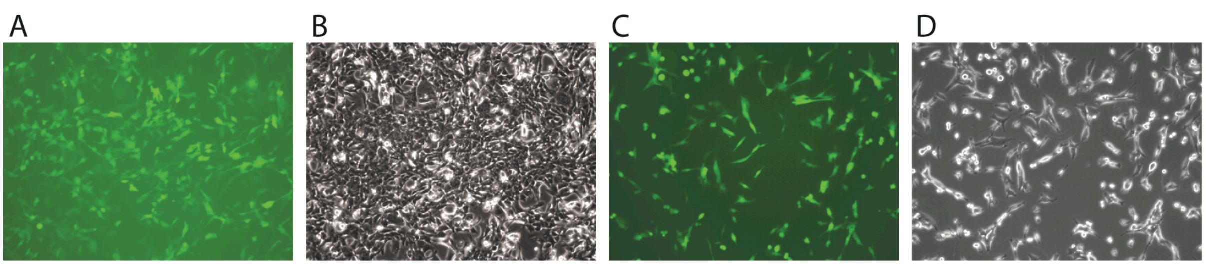
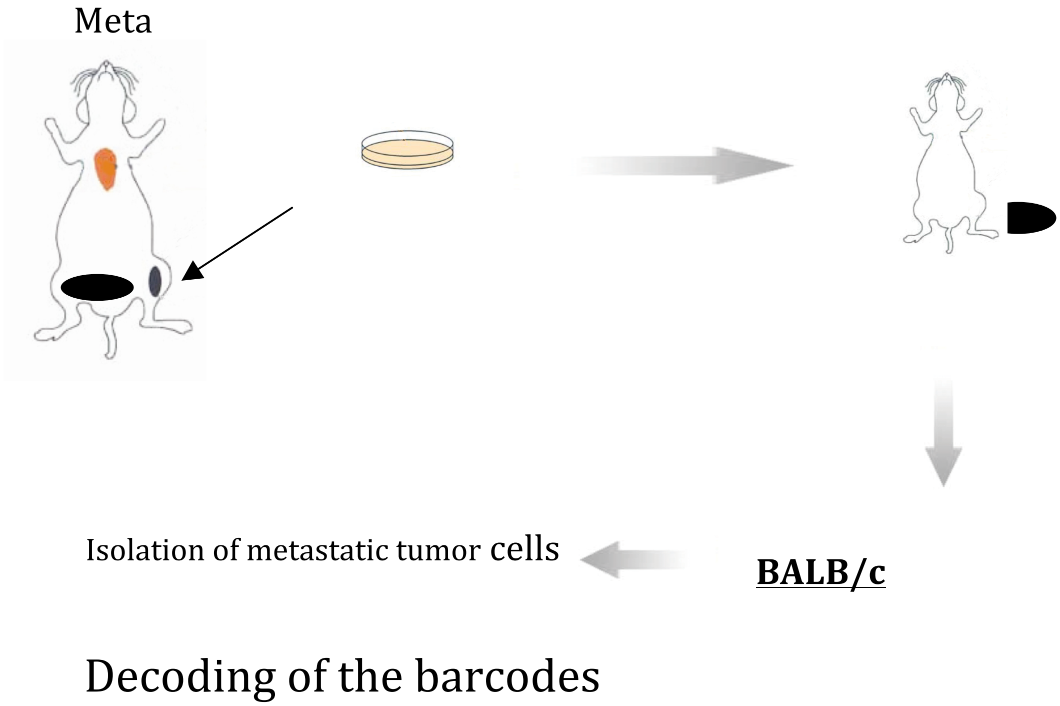



Figure 2. Images of TM40D-MB-Luc cells transduced with the GFP expressing lentivirus. (A, B) GFP and bright-field images of cells at 72 hours post infection without puromycin selection. (C, D) GFP and bright-field images of cells at 10 days post infection with puromycin selection.




### Key Research Accomplishments

- A library of lentiviral constructs containing a reporter GFP gene driven by EF1A promoter, as well as randomized 10-bp barcode sequences in both the 5' UTR and 3' UTR of the reporter.

- A lentivirus library carrying the above constructs.

- A library of mouse breast cancer cells transduced with the above lentivirus library.

- TM40D-barcoded TM40D-MB tumor cells were injected to BALB/c mice and breast tumor developed and were able to metastasized to lung and bone.

- We have used G418 to select for the neomycin resistant TM40D-MB barcoded cells from lung or bone, and harvested tumor cells in vitro.
- We have subcloned two metastatic cell lines (barcoded MCCs) and they will be decoded by Dr. Lahn's lab in the near future.

### Reportable Outcomes

We have developed a set of DNA vectors, lentivirus and cell lines during the reporting period.

TM40D-barcoded cells were isolated from metastatic tissues in mice and harvested and subcloned for the decoding of the barcodes by the Lahn laboratory.

### Conclusion

The comprehensive suite of reagents generated in the first reporting period, such as vector library, lentivirus library, and cell library, set the stage for utilizing these reagents in animal models of breast cancer metastasis in the last year of the project.

References: